APPSL-GFP Alzheimer's Lentivirus

Catalog No. SCR526

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Not for use in diagnostic procedures.

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Introduction

Amyloid-β (Aβ) plagues and neurofibrillary tangles are key pathological features observed in the brains of Alzheimer's patients. Familial, early onset forms of AD (FAD) are caused by autosomal dominant inherited genetic mutations and offer an opportunity to study the effects of key mutations on the disease's progression and pathology[1]. To date, approximately 200 FAD mutations in APP and/or PSEN1 have been reported[2]. Transgenic mouse models and human neurons harboring FAD mutations in PSEN-1 and/or APP are widely used as model systems and have provide important insights into the disease. Transgenic mouse models are able to recapitulate the loss in cognitive functions along with increased deposition of β-amyloid plagues, but are unable to demonstrate neurofibrillary tangles, one of the key pathological hallmarks of AD[2,3, 4]. Similarly human iPSC-derived neurons from FAD patients demonstrated increased levels of the pathogenic Aβ42 species and phosphorylated tau, but lack the characteristic amyloid-β plagues and neurofibrillary tangles[2, 3, 4].

Recently a 3D model using genetically engineered human neural stem cells that overexpress FAD mutations was reported to recapitulate the two pathological hallmarks of AD – β-amyloid plagues and neurofibrillary tangles[3,4]. Lentiviruses expressing FAD mutations in human APP with both the K670N/M671L (Swedish) and V717I (London) mutations (APPSL) and/or PSEN1 with the ΔE9 mutation (PSEN1(ΔE9)) and APPSL/PSEN1(ΔE9) along with fluorescent proteins as reporters for viral infection (see below), were used to transfect ReNcell VM human neural stem cells[3,4]. FACS was utilized to enrich for cells with the highest transgene expressions followed by encapsulation of the sorted cells in a 3D Matrigel culture system. After approximately 6 weeks of differentiation, aggregation of Aβ was observed. Robust accumulation of phosphorylated tau along neurofibrillary tangles was readily detectable after 10-14 weeks[3, 4].

MilliporeSigma's APPSL-GFP Lentivirus is a necessary reagent to set up the Alzheimer's 3D culture system. The lentivirus contains the APPSL FAD mutations and can be used to infect human neural cells. The TagGFP2 tag enables assessment of viral infectivity and allows FACS sorting of the highest expressing cells.

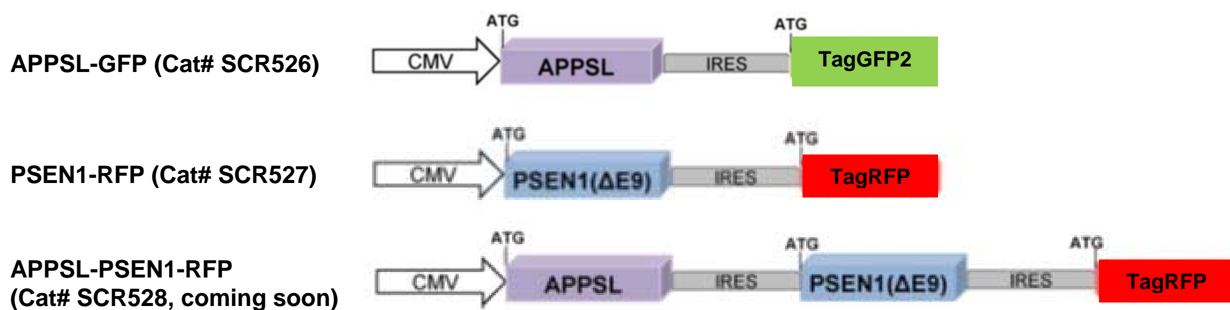

Figure 1. Polycistronic lentiviral vectors expressing FAD mutations. APPSL-GFP Lentivirus (Cat. No. SCR526) expresses the full length human β-amyloid precursor protein (APP_{695}) gene containing both the K670N/M671L (Swedish) and V717I (London) FAD mutations (APPSL) and the TagGFP2 separated by IRES sequences[3, 4]. PSEN1-RFP Lentivirus (Cat. No. SCR527) expresses the PSEN1 containing the ΔE9 FAD mutation (PSEN1(ΔE9)) and the TagRFP separated by the IRES sequences[3, 4]. APPSL-PSEN-RFP Lentivirus (Cat. No. SCR528) expresses both the APPSL and PSEN1 mutations and the TagRFP separated by the IRES sequences[3, 4].

Note: *PSEN1-RFP Alzheimer's Lentivirus is available separately as Cat. No. SCR527. APPSL-PSEN-RFP Lentivirus will be available soon as Cat. No. SCR528). Please check back for updates.*

Lentiviral particles were generated using the pPACKH1 Lentivector Packaging System at System Biosciences (SBI). www.systembio.com.

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Materials Provided

1. APPSL-GFP Alzheimer's Lentivirus: (Part No. CS222734) One (1) vial containing 15 µL of high titer lentiviral particles. Store at -80°C.

   ***Note**: For exact viral titer, refer to the label on the vial.*

Storage and Handling

Lentivirus is stable for at least 6 months when stored at -80°C. After first thaw, place immediately on ice and store in working aliquots to avoid further freeze thaws. Avoid freeze thaws as this will result in a decrease in the virus titer.

Important Safety Note:

- Replication-defective lentiviral vectors are not known to cause any diseases in humans or animals. However, lentiviruses can integrate into the host cell genome and thus pose some risk of insertional mutagenesis.
- Wear gloves when using this product. Avoid skin contact or ingestion of all reagents and chemicals used in this protocol.
- Lentivirus is a risk group 2 and should be handled under approved Biosafety Level 2 (BSL2) controls.

Materials Required But Not Provided

- ReNcell VM Human Neural Progenitor Cell Line (Cat No. SCC008) or other neural cell lines of interest.
- ReNcell NSC Maintenance Media (Cat. No. SCM005)
- ReNcell Neural Stem Cell Freezing Medium (Cat No. SCM007)
- EGF Protein, Human Recombinant Animal Free (Cat No. GF316)
- Basic Fibroblast Growth Factor, Human recombinant (bFGF; Cat No. GF003)
- Growth factor reduced Matrigel (Corning Cat No. 356230)
- Accumax™ Cell Detachment Solution (Cat. No. SCR006)
- Polybrene Infection / Transfection Reagent (Cat. No. TR-1003-G)

Overview of 3D Alzheimer's Culture Model

A complete step-by step protocol can be found in "A 3D human neural cell culture system for modeling Alzheimer's disease", *Nature Protocols* (2015) 10(7): 985 – 1006.

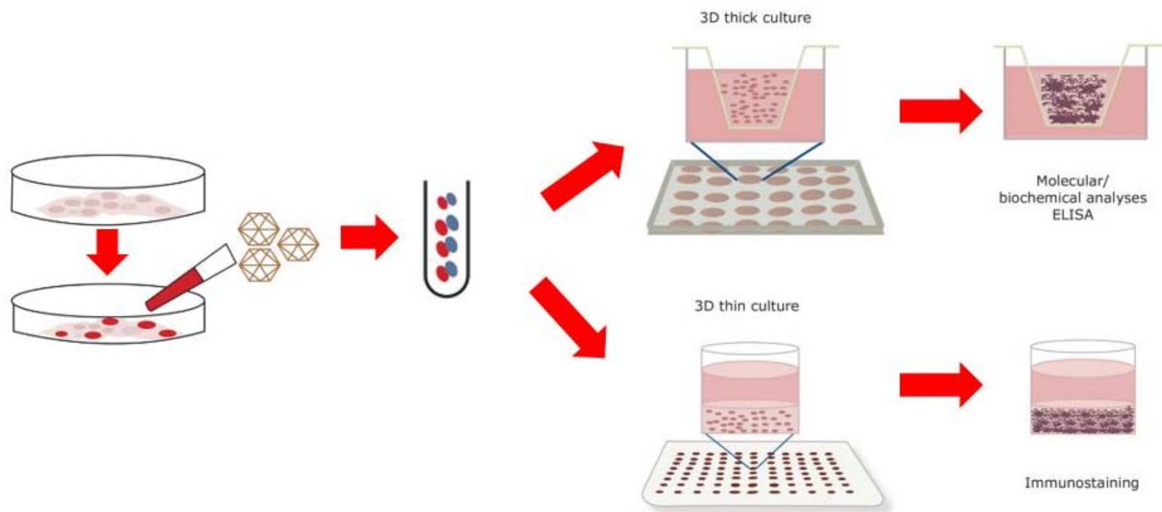

Figure 2. General overview for creating 3-dimensional cell culture models of Alzheimer's disease using ReNcell VM human neural progenitor cells. Breifly, ReNcell VM NSCs are transduced with either APP, PSEN or APP/PSEN expressing lentiviruses, positive cells are sorted and embedded into Matrigel matrix for 3D culture over multiple weeks.

Protocol for lentivirus infection

Important Note: The following protocol has been validated using ReNcell VM Neural Progenitor Cell Line (Cat. No. SCC008) and should be used as a general guideline. Modifications to the protocol may be needed to optimize viral tansduction of other neural cell lines. Depending on the cell types, a higher MOI or multiple viral transductions may be required.

Before Starting:

1. Determine the optimal plating density of target cells. The optimal plating density is defined as the number of cells that should be plated at Day 0 in order to have the cells reach 90% confluence by day 3. Plate out a range of cell numbers from 1 x 10^5 to 1 x 10^6 cells per well of a 6-well plate. Culture medium should be the same as that used to maintain the target cells in a proliferative state. Volume should be 2 mL per well of a 6-well plate. For each cell number range, a control well should be set aside for counting the number of cells on the day of transduction. The number of cells to be seeded at Day 0 may vary depending on the cell types due to differences in cell sizes, morphology and rate of proliferation. Whenever possible, use lower passage cells as they possess higher proliferative potential than higher passaged cells.

Day 0

2. Plate optimal density of target cells as determined from step 1. A control well should be set aside for counting the cell numbers on the day of transduction. Incubate overnight in a 37°C, 5% CO_2 incubator.

   If using ReNcell VM: The optimal plating density for ReNcell VM is 2 x 10^5 cells per well of a 6-well plate. Culture medium is ReNcell NSC Maintenance medium (SCM005) supplemented with 20 ng/mL EGF (Cat. No. GF316) and 20 ng/mL FGF-2 (Cat. No. GF003). Total volume = 2 mL per well.

Day 1

3. Before transduction, count the number of cells in the control well. This cell count is used to calculate the volume of virus needed to achieve a target MOI.

4. Using the equation provided below, determine the volume of virus required to achieve a high transduction efficiency of 80 – 90% transgene expressing cells after 72 hours (3 days). **Please make note of the titer as it may vary slightly from lot to lot.**

   Note: The percentage of GFP expression cells will not reach maximal levels at 24 hours. Please monitor at 72 hours (3 days) instead.

   If using ReNcell VM: As a general guideline, an MOI of 20 used to transduce 3 x 10^5 ReNcell VM cells (cell count at Day 1), resulted in 60-80% cells visibly expressing the GFP fluorescent protein by day 3. The MOI can be increased to achieve >90% transduction efficiency.

   $$\text{Virus volume (µL) required} = \frac{\text{Number of cells seeded (from step 1)}}{\text{Virus Titer (IFU/mL)}} \times \frac{\text{Desired MOI}}{1\text{ mL}} \times 1000\text{ µL}$$

   Example: If the number of cells in the well at the time of transduction is 3 x 10^5, the viral titer is 1 x 10^9 IFU/mL and a desired MOI is 20, then the volume of virus required can be calculated as below. ***Note***: Optimal MOI must be determined empirically and may change with different cell lines and different FAD expressing lentiviruses.

   $$\frac{3 \times 10^5\text{ cells}}{1\times10^9\text{ IFU/mL}} \times \frac{20}{\text{mL}} \times 1000\text{ µL} = 6\text{ µL virus required for 1 well of a 6-well plate}$$

5. Thaw the vial of APPSL-GFP Alzheimer's Lentivirus (~15 µL) on ice. Quickly centrifuge the vial to spin down the contents. Keep the virus on ice and proceed immediately to the next step.

6. Replace the medium from each well with 2 mL fresh ReNcell NSC Maintenance Medium containing 20 ng/mL FGF2 and 20 ng/mL EGF.

7. Dilute 1 µL of Polybrene transfection reagent into 9 µL of sterile distilled water to create a 1:10 dilution. Add 10 µL of the diluted Polybrene transfection reagent to each well to be transduced. Final polybrene concentration should be 5 µg/mL.

8. Add the required volume of thawed virus (from Step 4) directly to the wells containing the attached cells of interest. Gently rock the plate from side to side to thoroughly mix the virus onto the target cells. Incubate the plate overnight in a 37°C, 5% CO_2 incubator.

Day 2

9. Gently wash the cells twice with 2 mL 1X PBS per well. Be careful to not detach the cells during washes. Aspirate after each wash.

10. Replace with 2 mL fresh ReNcell NSC maintenance Medium per well. Monitor cell morphology daily.

11. ***Optional:*** Perform a 2nd virus infection by repeating Steps 4 – 7 to increase the transduction efficiency. ***Note:*** It is important to achieve >90% transduction efficiency after 3 days to be able to do the subsequent FAC-sorting of transgene positive cells and perform the 3D culture protocol.

Day 3 – 4

12. Replace with 2 mL fresh ReNcell NSC maintenance media per well. Cell confluence should increase over time. Observe transgene expression using a fluorescent microscope 48 hours after infection. Evaluate the infection efficiency by flow cytometry at Days 3 – 4.

13. Sort transgene positive cells as soon as possible. If cells are confluent, passage to a matrigel coated T25 flask. ***Note:*** Expression of GFP is expected to decrease rapidly with time, especially after the first passage (see below) and thus it is important to sort cells soon or freeze down the P0 cells to avoid further passages.

14. For 3D cell culture, refer to "A 3D human neural cell culture system for modeling Alzheimer's disease", *Nature Protocols* (2015) 10(7): 985 – 1006[4].

    Note: The presence and degree of Aβ and phosphorylated tau aggregation may depend on the neural cell type, the passage number, the number of cells seeded and the degree of enrichment of the sorted cell population[3, 4].

Data Analysis

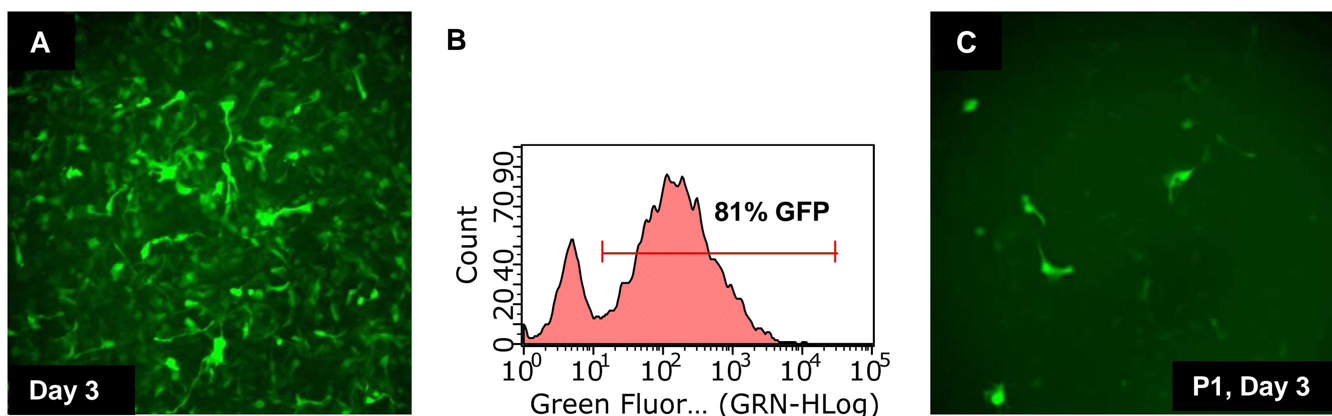

Figure 1. Fluorescence images of ReNcell VM infected with APPSL-GFP Alzheimer's Lentivirus, MOI =20. By day 3 post viral transduction, approximately 81% ReNcell VM express the transgenes (**A, B**). Cells were passaged at days 3 -4. Three days after passage, the fluorescence signal is significantly diminished (**C**). Cells were not sorted.

References

1. Ryan NS, Rossor MN (2010) Correlating familial Alzheimer's disease gene mutations with clinical phenotype. *Biomark Med.* 4(1): 99-112.

2. D'Avanzo C, Aronson J, Kim YH, Choi SH, Tanzi RE, Kim DY (2015) Alzheimer's in 3D culture: challenges and perspectives. *Bioassays* 37(10): 1139-1148.

3. Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, Klee JB, Zhang C, Wainger BJ, Peitz M, Kovacs DM, Woolf CJ, Wagner SL, Tanzi RE, Kim DY (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* 515(7526): 274-278.

4. Kim YH, Choi SH, D'Avanzo C, Hebisch M, Sliwinski C, Bylykbashi E, Washicosky KJ, Klee JB, Brüstle O, Tanzi RE, Kim DY (2015) A 3D human neural cell culture system for modeling Alzheimer's disease. *Nature Protocol* 10(7): 985-1006.

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